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L1: Entry 1 of 1 File: USPT Aug 5, 1997

DOCUMENT-IDENTIFIER: US 5654413 A
TITLE: Compositions for sorting polynucleotides

DEPR:
When microparticles are used as supports, repertoires of oligonucleotide tags and tag complements are preferably generated by subunit-wise synthesis via "split and mix" techniques, e.g. as disclosed in Shortle et al, International patent application PCT/US93/03418. Briefly, the basic unit of the synthesis is a subunit of the oligonucleotide tag. Preferably, phosphoramidite chemistry is used and 3' phosphoramidite oligonucleotides are prepared for each subunit in a minimally cross-hybridizing set, e.g. for the set first listed above, there would be eight 4-mer 3'-phosphoramidites. Synthesis proceeds as disclosed by Shortle et al or in direct analogy with the techniques employed to generate diverse oligonucleotide libraries using nucleosidic monomers, e.g. as disclosed in Telenius et al, Genomics, 13: 718-725 (1992); Welsh et al, Nucleic Acids Research, 19: 5275-5279 (1991); Grothues et al, Nucleic Acids Research, 21: 1321-1322 (1993); Hartley, European patent application 90304496.4; Lam et al, Nature; 354: 82-84 (1991); Zuckerman et al, Int. J. Pept. Protein Research, 40: 498-507 (1992); and the like. Generally, these techniques simply call for fine application of mixtures of the activated monomers to the growing oligonucleotide during the coupling steps.

DEPR:
As mentioned above, tag complements may also be synthesized on a single (or a few) solid phase support to form an array of regions uniformly coated with tag complements. That is, within each region in such an array the same tag complement is synthesized. Techniques for synthesizing such arrays are disclosed in McGall et al, International application PCT/US93/03767; Pease et al, Proc. Natl. Acad. Sci., 91: 5022-5026 (1994); Southern and Maskos, International application PCT/GB89/01114; Maskos and Southern (cited above); Southern et al, Genomics, 13: 1008-1017 (1992); and Maskos and Southern, Nucleic Acids Research, 21: 4663-4669 (1993).

DEPR:
The tagging system of the invention can be used with single base sequencing methods to sequence polynucleotides up to several kilobases in length. The tagging system permits many thousands of fragments of a target polynucleotide to be sorted onto one or more solid phase supports and sequenced simultaneously. In accordance with a preferred implementation of the method, a portion of each sorted fragment is sequenced in a stepwise fashion on each of the many thousands of loaded microparticles which are fixed to a common substrate--such as a microscope slide--associated with a scanning system, such as that described above. The size of the portion of the fragments sequenced depends of several factors, such as the number of fragments generated and sorted, the length of the target polynucleotide, the speed and accuracy of the single base method employed, the number of microparticles and/or discrete regions that may be monitored simultaneously; and the like. Preferably, from 12-50 bases are identified at each microparticle or region; and more preferably, 18-30 bases are identified at each microparticle or region. With this information, the sequence of the target polynucleotide is determined by collating the 12-50 base fragments via their overlapping regions, e.g. as described in U.S. Pat. No. 5,002,867. The following references provide additional guidance in determining the portion of the fragments that must be sequenced for successful reconstruction of a target polynucleotide of a given length: Drmanac et al, Genomics, 4: 114-128 (1989); Bains, DNA Sequencing and Mapping, 4: 143-150 (1993); Bains, Genomics, 11: 294-301 (1991); Drmanac et al, J. Biomolecular Structure and Dynamics, 8: 1085-1102 (1991); and Pevzner, J. Biomolecular Structure and Dynamics, 7: 63-73

1085-1102 (1991); and Pevzner, J. Biomolecular Structure and Dynamics, 7: 63-73 (1989). Preferably, the length of the target polynucleotide is between 1 kilobase and 50 kilobases. More preferably, the length is between 10 kilobases and 40 kilobases.